

WHAT IS CLAIMED IS:

1. An isolated or purified nucleic acid molecule comprising a nucleotide sequence which codes for a pyruvate carboxylase enzyme of SEQ ID NO:19, wherein said pyruvate carboxylase enzyme contains at least one mutation which desensitizes said pyruvate carboxylase enzyme to feedback inhibition by aspartic acid selected from the group consisting of:

- a) methionine at position 1 is replaced with a valine,
- b) glutamic acid at position 153 is replaced with an aspartic acid,
- c) alanine at position 182 is replaced with a serine,
- d) alanine at position 206 is replaced with a serine,
- e) histidine at position 227 is replaced with an arginine,
- f) alanine at position 452 is replaced with a glycine, and
- g) aspartic acid at position 1120 is replaced with a glutamic acid.

2. An isolated or purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- a) the nucleotide sequence encoding amino acids 1 to 1157 of SEQ ID NO:2;
- b) the nucleotide sequence encoding amino acids 1 to 1140 of SEQ ID NO:4;
- c) a nucleotide sequence encoding the amino acid sequence encoded by the DNA contained in Deposit Number NRRL B-30293; and
- d) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b) or (c).

3. The nucleic acid molecule of claim 2, comprising the nucleotide sequence of SEQ ID NO:1.

4. The nucleic acid molecule of claim 2, comprising the nucleotide sequence of SEQ ID NO:3.

5. A vector comprising:

- a) the nucleic acid molecule of claim 1 or 2; and
- b) at least one marker gene.

6. The vector of claim 5, further comprising a functional *Corynebacterium* replication origin.

7. A method for producing a host cell comprising introducing the vector of claim 5 into a host cell.

8. A host cell comprising the vector of claim 5.

9. A method of producing an amino acid, comprising:

- a) culturing the host cell of claim 8, in a suitable media; and
- b) separating said amino acid from said medium.

10. The method of claim 9, wherein said amino acid is selected from the group consisting of: L-lysine, L-threonine, L-methionine, L-isoleucine, L-glutamic acid, L-arginine and L-proline.

11. The method of claim 10, wherein said amino acid is L-lysine.

0597497 101204

12. A method for replacement of a wild-type pyruvate carboxylase gene, with a feedback resistant pyruvate carboxylase gene, in a *Corynebacterium glutamicum* host cell comprising the steps of:

a) replacing a genomic copy of said wild-type pyruvate carboxylase gene with a selectable marker gene through homologous recombination to form a first recombinant strain; and

b) replacing said selectable marker gene of step (a) in said first recombinant strain, with said feedback resistant pyruvate carboxylase gene through homologous recombination to form a second recombinant strain;

wherein said homologous recombination in steps (a) and (b) occurs between said host cell and the vector of claim 5.

13. A host cell produced by the method of claim 12.

14. A method of producing an amino acid, comprising:

a) culturing the host cell of claim 13 in a suitable medium; and

b) separating said amino acid from said medium.

15. The method of claim 14, wherein said amino acid is selected from the group consisting of: L-lysine, L-threonine, L-methionine, L-isoleucine, L-glutamic acid, L-arginine and L-proline.

16. The method of claim 15, wherein said amino acid is L-lysine.

17. An isolated or purified polypeptide comprising the amino acid sequence of the polypeptide encoded by the DNA plasmid encoding pyruvate carboxylase contained in Deposit Number NRRL B-11474, the amino acid sequence of SEQ ID NO:2 or the amino acid sequence of SEQ ID NO:4.